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# Heritability and Whole Genome Linkage of Pulse Pressure in Chinese Twin Pairs

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Elevated pulse pressure is associated with cardiovascular disorders and mortality in various populations. The genetic influence on pulse pressure has been confirmed by heritability estimates using related individuals. Recently, efforts have been made in mapping genes that are linked to the phenotype. We report results on our heritability and linkage study conducted on the Chinese population in mainland China where cardiovascular and cerebrovascular diseases are becoming the leading cause of death. A total of 630 pairs of middle-aged Chinese twins were collected for heritability analysis, from which 63 dizygotic twin pairs were randomly selected for genome-wide linkage analysis using Affymetrix 6.0 SNP array. Regression analysis reconfirmed the significant effects of age, sex, and BMI on pulse pressure. Comparison of twin models suggested the parsimonious AE model as the best model with a heritability estimate of 0.45. Genome-wide non-parametric linkage analysis identified three significant linkage peaks on chromosome 11 (lod score 4.06 at 30.5 cM), chromosome 12 (lod score 3.97 at 100.7 cM), and chromosome 18 (lod score 4.01 at 70.7 cM) with the last two peaks closely overlapping with linkage peaks reported by two American studies. In addition, multiple regions with suggestive linkage were identified with many of them overlapping with published linkage regions. Our results provide both epidemiological and molecular genetic evidence for the genetic dissection of pulse pressure in the Chinese population, which could help in fine mapping and in characterizing genes that are involved in the regulation of pulse pressure.

■ **Keywords:** Chinese twins, pulse pressure, heritability, linkage analysis

Pulse pressure (PP) measures the change in blood pressure during a contraction of the heart. Elevated pulse pressure has been associated with cardiovascular disorders and mortality (Dart & Kingwell, 2001). Several anthropometric factors have been shown to affect PP; for example, aging results in increased stiffness and loss of elasticity in the aorta and major artery leading to increased PP (Franklin et al., 1997), body-size (thus gender) affects PP through altering magnitude and timing of wave reflection (London et al., 1995; Smulyan et al., 1998), and recently age and gender effects have been shown to be cross-cultural (Skurnick et al., 2010).

Besides anthropometric factors, genetic epidemiology studies using family and twin data have provided verifiable evidence for a low to modest genetic contribution in pulse pressure with heritability estimates ranging from 0.13 using twins in the UK (Snieder et al., 2000), 0.21 (Pilia et al., 2006) and 0.24 (van Rijn et al., 2007) in European pedigrees, to 0.35 and 0.54 in American Caucasian (Mitchell et al., 2005) and African families (Bochud et al., 2005). These results indicate that, similar to all other complex traits, the etiology of PP is complicated involving both genetic and environmental dissections (Turner & Boerwinkle, 2003).

The existence of a genetic contribution has warranted efforts to elucidate genes affecting pulse pressure, especially in recent years. For example, genetic marker-based linkage studies have identified multiple loci harboring susceptibility genes for PP in different populations including Caucasians

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(Bielinski et al., 2005; Camp et al., 2003; DeStefano et al., 2004; Mitchell et al., 2005), Mexicans (Atwood et al., 2001), American Indians (Franceschini et al., 2008), and African Americans (Sherva et al., 2007). The verification of susceptibility loci requires replication studies within and even across populations in order to consolidate linkage results. This is especially important given the fact that inconsistent linkage results have been reported by different studies (Turner & Boerwinkle, 2003).

This paper reports results from the first twin-based genetic epidemiological study on pulse pressure in the Chinese population. Results from our study will be compared with those reported in the literature and the significance of our findings will be discussed.

## Material & Methods

### Twin Samples

All twins were taken from the most recent wave of a twin sample for a heritability study on multiple phenotypes associated with the metabolic syndrome, conducted by the Qingdao Twin Registry at Qingdao Center for Disease Control and Prevention in 2008. Twins were sampled through the local disease control network and residence registry. After informed consent was obtained, twins were invited to a clinical investigation if both co-twins were alive. Those who were pregnant, breastfeeding, had known diabetes and/or cardiovascular disease, or were taking weight-reducing medication within one month, were excluded, and incomplete twin pairs were discharged. Zygosity of like-sex twin pairs was determined by DNA testing using 16 short tandem repeat DNA markers. A total of 389 pairs of monozygotic (MZ) and 241 pairs of dizygotic (DZ) twins were sampled for heritability analysis, among which were 63 pairs of DZ twins randomly selected for genome-wide linkage analysis.

### Phenotypes

Blood pressure was taken by a standard procedure using mercurial table stand model sphygmomanometer. Systolic blood pressure (SBP) was calculated as Korotkof phase I (appearance of sound), and diastolic blood pressure (DBP) as Korotkof phase V (disappearance of sound). Pulse pressure was calculated as the difference between systolic and diastolic blood pressures. BMI (kg/m$^2$) was obtained according to measured values of height and weight, by taking the subject's weight (in kilograms) and dividing by the subject's height (in meters) squared. All measurements above three standard deviations below or over the mean were assigned as missing values. In all subsequent analyses, we use the natural log transformation of PP to ensure normal or approximate normal distribution.

### DNA Samples and Genotyping

Whole blood samples were taken for extracting leukocytes for DNA purification and genotyping using standard procedures. We used the Affymetrix Genome-Wide Human SNP Array 6.0 featuring 1.8 million genetic markers among which were more than 906,600 single nucleotide polymorphisms (SNPs) enabling high resolution genome-wide analysis. Detailed information about the array can be found at the manufacturer's website (www.affymetrix.com). Genotyping was performed by the Affymetrix service provider, Shanghai Biochip, in China using purified DNA samples. Genotyping was high quality with SNP calling rate ranges from 93.4% to 99%.

### Generalized Estimating Equations for Twins

It has been shown that age, sex, and BMI are important factors affecting pulse pressure (Skurnick et al., 2010). With our data, we first want to test the effects of these factors in Chinese subjects and then adjust PP to remove the influences by these factors in the subsequent analyses. Since our twin samples were correlated samples, we introduced the generalized estimating equations (GEE) with an exchangeable working correlation matrix (Zeger & Liang, 1986). GEE was applied to MZ and DZ twins separately considering differences in their genetic correlations, which could result in different working correlation matrices. GEE models were fitted using the free R package *gee* (http://cran.r-project.org/web/packages/gee/index.html).

### Intra-Class Correlation Coefficients (ICC)

After fitting the GEEs, residuals are kept for calculating ICC to show twin correlation on pulse pressure after adjusting for age, sex, and BMI. Following the definition (McGraw & Wong, 1996), the statistic is calculated as $ICC = \frac{\mathrm{MSB}-\mathrm{MSW}}{\mathrm{MSB}+\mathrm{MSW}}$. Here MSW is the within-pairs mean square and MSB the between-pairs mean square of the adjusted PP. ICC was estimated using the free R package *psy* (http://cran.r-project.org/web/packages/psy/index.html). Significance tests on statistical differences between MZ and DZ twin correlation were performed first by transforming an ICC ($r$) using $r' = (0.5)\log_e|\frac{1+r}{1-r}|$ and then computing the test statistic as $z = \frac{r'_{\mathrm{mz}} - r'_{dz}}{\sqrt{\frac{1}{n_{mz}-3}+\frac{1}{n_{dz}-3}}}$, where $r'_{\mathrm{mz}}$ and $r'_{\mathrm{dz}}$ are the transformed coefficients, and $n_{\mathrm{mz}}$ and $n_{\mathrm{dz}}$, the number of pairs for MZ and DZ twins. $P$ values were obtained by referring the test statistic $z$ to a standard normal distribution with one degree of freedom.

### Heritability Estimation

We introduced the structural equation model to fit univariate genetic models to our twin data. The univariate model included additive genetic (A), common environmental (C), dominant genetic (D), and unique environmental (E) effects. As the effects of C and D are confounded in twin data (Rijsdijk & Sham, 2002), two full models, i.e., the ACE and ADE models, were fitted separately to the data. Discrimination of the two models was made using Akaike's Information Criterion (AIC) with the lowest AIC for the best model. The best fitting and most parsimonious model




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**TABLE 1**

**Descriptive, Regression, and Correlation Analyses**

| | MZ (389 pairs) | | DZ (241 pairs) | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| Age, yrs | 37.36 | 9.29 | 36.26 | 9.29 |
| BMI, kg/m$^2$ | 23.89 | 3.04 | 23.82 | 3.16 |
| SBP, mmHg | 118.08 | 15.52 | 120.05 | 15.30 |
| DBP, mmHg | 80.07 | 11.15 | 81.43 | 10.75 |
| PP, mmHg | 38.80 | 8.77 | 39.44 | 9.32 |
| GEE for PP | Reg. coef. | *p* value | Reg. coef. | *p* value |
| Age, year | 0.003 | 3.85e-04 | 0.004 | 1.69e-03 |
| Sex, m = 1; f = 2 | −0.099 | 3.62e-10 | −0.081 | 7.99e-05 |
| BMI, kg/m$^2$ | 0.222 | 1.94e-04 | 0.226 | 4.69e-03 |
| ICC* | *r*MZ | *p* value | *r*DZ | *p* value |
| | 0.42 | 5.00e-07 | 0.19 | 3.06e-03 |

Note: *ICCs in MZ and DZ twins are statistically significantly different with a *p* value of 1.90e-03 ($Z = 3.10$).

from the nested models of either ACE or ADE was selected based on the likelihood ratio test for nested models and AIC for competing models not nested. The fitting of twin models was all performed using the free software package Mx (Neale et al., 2003).

### Genome-Wide Linkage Analysis

We applied the variance components method for non-parametric linkage analysis (Marlow, 2002) to our genome-wide SNP marker data on DZ twins. Data were analyzed with the free linkage software package, Merlin (Abecasis et al., 2002). Before linkage analysis, our SNP genotype data were first processed for genotyping errors using the error-detection procedure in Merlin (*–error*). The detected unlikely genotypes were removed from the data using the Merlin command *pedwipe*. The variance components (Amos, 1994) (*–vc*) procedure was used to test for linkage for pulse pressure with age, sex, and BMI incorporated as covariates.

Although our high density SNP map increases linkage information content which is important in non-parametric linkage analysis (Evans & Cardon, 2004), on the other hand, it could also result in linkage disequilibrium (LD) among adjacent SNPs. When parental phase information is missing, LD can lead to overestimation of the number of alleles shared by identical-by-descent (IBD) and thus inflate the lod score estimates (Cho & Dupuis, 2009). To accommodate LD, Merlin organizes correlated SNPs into clusters and assumes linkage equilibrium between the clusters and no recombination within clusters. This is done using the Merlin procedure *–cluster* and defining a threshold for pairwise marker-marker correlation $r^2$. In our analysis, we introduce a stringent threshold of 0.1. According to a simulation study by Cho and Dupuis (Cho & Dupuis, 2009), such a threshold can efficiently eliminate the lod score inflation due to LD.

Genome-wide significance of the identified linkage peaks were assessed by applying the threshold criterion provided by Lander and Kruglyak (Lander & Kruglyak, 1995) with a score of 2.2 for suggestive and 3.6 for genome-wide significant linkages.

## Results

### Basic Statistics

The basic statistics for all subjects are shown in Table 1 for MZ and DZ twins separately. The mean age for all twins was approximately 37 years with a mean BMI around 24 kg/m$^2$, mean SBP around 119 mmHg, mean DBP around 81 mmHg, and mean PP around 39 mmHg. In both GEE models for MZ and DZ twins, age showed a highly significant ($p < .002$) positive correlation with PP indicating a slow but steady increase of PP with increasing age, when sex and BMI are balanced. Sex has very highly significant negative correlations consistent in both MZ and DZ twins meaning that, for given age and BMI, females tend to have lower PP than males. The effect for BMI is also highly

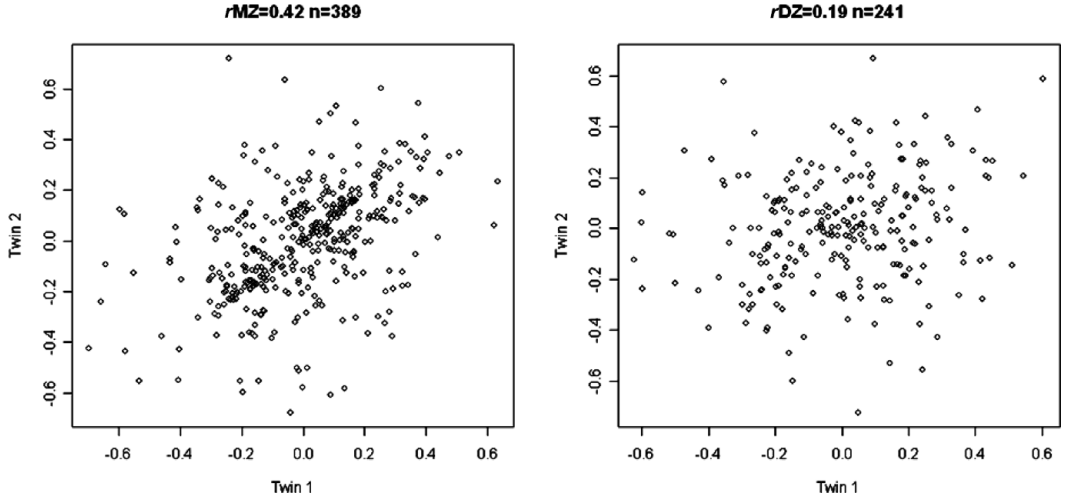


**FIGURE 1**

**Scatter plot for the residuals of log PP from GEE models fitted to MZ (left) and DZ (right) twins. MZ twins are more closely correlated than the DZ twin suggesting the existence of genetic contributions to PP.**




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**TABLE 2**

**Estimates (95% Confidence Interval) and Model Performances for ACE, ADE, and Their Nested Models**

| | A | C, D | E | AIC | -2LL | Δ-2LL | *p* value |
|---|---|---|---|---|---|---|---|
| ACE | 0.45 (0.21,0.52) | 0.00 (0.00,0.21) | 0.55 (0.48,0.63) | –2844.77 | –274.77 | | |
| AE | 0.45 (0.37, 0.52) | | 0.55 (0.48,0.63) | –2846.77 | –274.77 | 0.00 | 1.00 |
| CE | | 0.36 (0.29,0.42) | 0.64 (0.58,0.71) | –2834.23 | –262.23 | 12.54 | 3.99e-04 |
| E | | | 1.00 | –2751.81 | –177.81 | 96.96 | 7.07e-23 |
| ADE | 0.41 (0.00,0.52) | 0.05 (0.00,0.50) | 0.54 (0.47,0.62) | –2844.81 | –274.81 | | |
| AE | 0.45 (0.37, 0.52) | | 0.55 (0.48,0.63) | –2846.77 | –274.77 | 0.04 | 0.84 |
| DE | | 0.00 | 1.00 | –2751.81 | –177.81 | 97.00 | 6.93e-23 |
| E | | | 1.00 | –2751.81 | –177.81 | 97.00 | 6.93e-23 |

**TABLE 3**

**Significant and Suggestive Linkage Peaks for Pulse Pressure**

| Chromosome Location | Closest marker | Ref SNP | LOD score | Point *p* value | Genome significance | Published reference# |
|---|---|---|---|---|---|---|
| *Chromosome 1* | | | | | | |
| 90.6 cM | SNP_A-2061574 | rs11584915 | 3.05 | 9.0e-05 | No | |
| 196.9 cM | SNP_A-8711523 | rs12118925 | 3.54 | 3.0e-05 | No | 17 |
| *Chromosome 3* | | | | | | |
| 32 cM | SNP_A-2179383 | rs9869761 | 2.86 | 1.4e-04 | No | 12 |
| 93.9 cM | SNP_A-8508966 | rs1387735 | 2.50 | 3.0e-04 | No | |
| 152 cM | SNP_A-2051216 | rs838610 | 3.60 | 2.0e-05 | No | |
| *Chromosome 4* | | | | | | |
| 116 cM | SNP_A-1916183 | rs2850413 | 3.08 | 8.0e-05 | No | |
| *Chromosome 5* | | | | | | |
| 83.3 cM | SNP_A-2158948 | rs33417 | 2.57 | 3.0e-04 | No | |
| *Chromosome 6* | | | | | | |
| 78.3 cM | SNP_A-8667531 | rs9357726 | 2.75 | 2.0e-04 | No | 12 |
| 143.9 cM | SNP_A-1941963 | rs9494756 | 3.19 | 6.0e-05 | No | |
| *Chromosome 7* | | | | | | |
| 57.7 cM | SNP_A-8399705 | rs6973458 | 3.24 | 6.0e-05 | No | |
| 74.4 cM | SNP_A-4247483 | rs10270531 | 3.41 | 4.0e-05 | No | 13; 14 |
| 102.5 cM | SNP_A-8635038 | rs7781900 | 3.18 | 7.0e-05 | No | |
| *Chromosome 8* | | | | | | |
| 26.5 cM | SNP_A-8293021 | rs17216024 | 2.79 | 2.0e-04 | No | |
| *Chromosome 9* | | | | | | |
| 159.5 cM | SNP_A-8464500 | rs11103695 | 3.49 | 3.0e-05 | No | |
| *Chromosome 10* | | | | | | |
| 134.5 cM | SNP_A-1832075 | rs7067647 | 2.80 | 2.0e-04 | No | |
| *Chromosome 11* | | | | | | |
| **30.5 cM** | **SNP_A-8636879** | **rs11603765** | **4.06** | **1.0e-05** | **Yes** | |
| *Chromosome 12* | | | | | | |
| **100.7 cM** | **SNP_A-8445857** | **rs2085866** | **3.97** | **1.0e-05** | **Yes** | 12 |
| *Chromosome 15* | | | | | | |
| 14.8 cM | SNP_A-8688089 | rs12592159 | 2.70 | 2.0e-04 | No | |
| 115.6 cM | SNP_A-8676706 | rs7177487 | 2.32 | 5.0e-04 | No | 14 |
| *Chromosome 16* | | | | | | |
| 3.1 cM | SNP_A-2181571 | rs4984954 | 2.87 | 1.4e-04 | No | |
| *Chromosome 17* | | | | | | |
| 75.3 cM | SNP_A-1842000 | rs17645899 | 3.32 | 5.0e-05 | No | |
| *Chromosome 18* | | | | | | |
| **70.7 cM** | **SNP_A-2005638** | **rs2576052** | **4.01** | **1.0e-05** | **Yes** | 13 |
| *Chromosome 20* | | | | | | |
| 86.1 cM | SNP_A-2133602 | rs16997979 | 2.38 | 5.0e-04 | No | |
| *Chromosome 22* | | | | | | |
| 5.6 cM | SNP_A-1970673 | rs5746419 | 3.04 | 9.0e-05 | No | |

significant with positive correlation in both MZ and DZ twins suggesting that when age and sex are fixed, PP increases with increasing BMI. Figure 1 plots the residuals (i.e., PP adjusted for age, sex, and BMI) from the GEE models for MZ and DZ twins. As can be seen in the figure, MZ twins exhibit an obvious higher correlation compared to DZ twins. The intra-pair correlation for the adjusted PP is shown in the bottom of Table 1 with ICCs of 0.42 for MZ ($p = 5e\text{-}07$) and 0.19 for DZ ($p = 3.06e\text{-}03$) twins, both are statistically highly significant. ICCs in MZ and DZ twins are significantly different with a $p$ value of .002 ($z$ score = 3.10, df = 1).

## Heritability Estimates

As the ICC for MZ twins is more than double the ICC for DZ twins, both ACE and ADE models were fitted to PP with age, sex, and BMI incorporated as covariates. Table 2 shows the results for the two models together with their corresponding nested models. Between the two competing full models, the ADE model slightly outperformed the ACE




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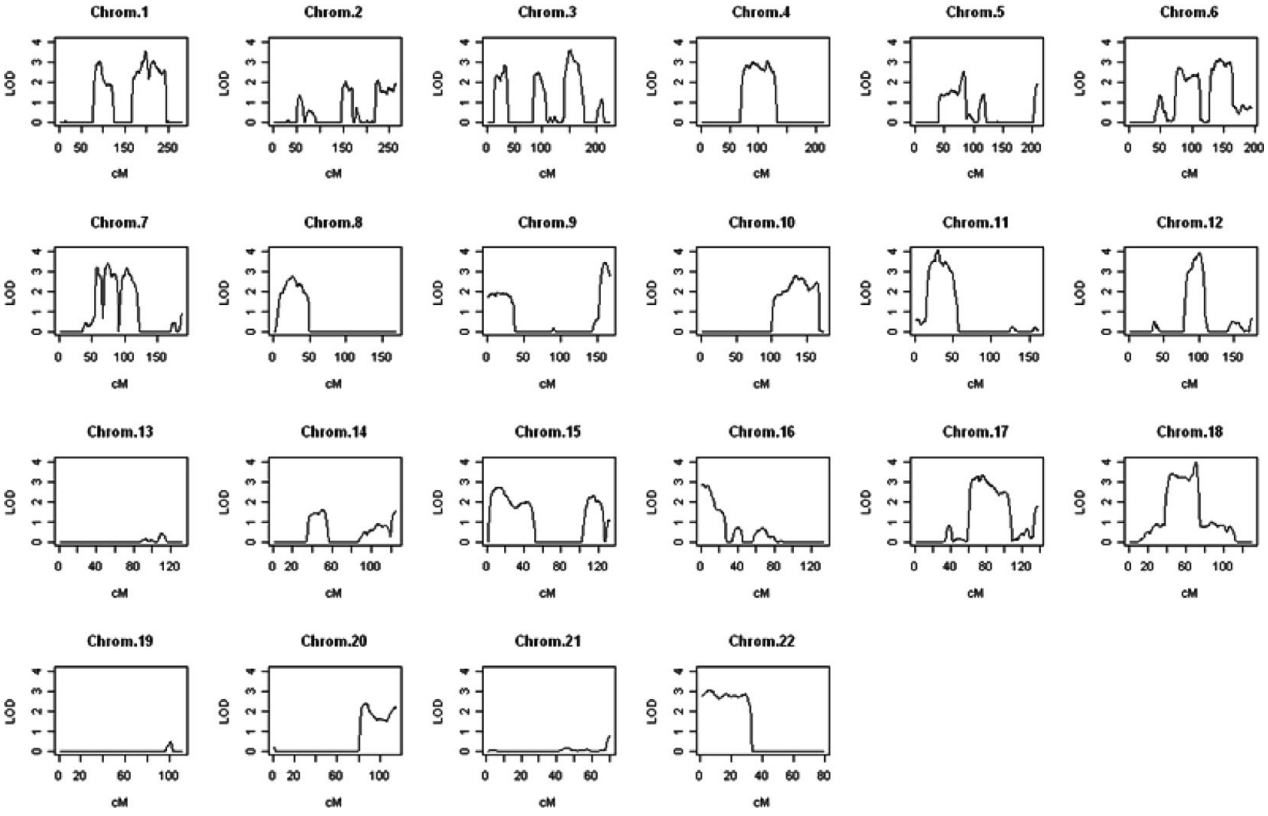


**FIGURE 2**

**Linkage results for genome-wide scan by chromosomes. Multiple suggestive linkage peaks were found on different chromosomes except chromosomes 2, 13, 14, 19, and 21.**

model according to their AICs (AIC for ADE model slightly more negative than AIC for ACE model). However, based on the likelihood ratio test and AICs, the nested parsimonious AE model for both ACE and ADE models was suggested as the best model with an estimate for additive genetic effect that accounts for 45% of the total variance in PP (95% CI 0.37–0.52). Performances for all other nested models were very significantly worse than that of their full models.

### Genome-Wide Linkage Analysis

With age, sex, and BMI incorporated as covariates, our whole genome linkage scan (Table 3) identified three significant linkage peaks at chromosome 11 (lod score of 4.06, linkage region from 26–33 cM), chromosome 12 (lod score of 3.97, from 93–104 cM) and chromosome 18 (lod score of 4.01, from 68–72 cM) (Figures 2 and 3), together with multiple suggestive linkage peaks (Figure 2) with lod scores over 3 on chromosome 1 (3.05 from 81–100 cM, 3.54 from 170–242 cM), chromosome 3 (3.6 from 141–170 cM), chromosome 4 (3.08 from 72–128 cM), chromosome 6 (3.19 from 129–163cM), chromosome 7 (3.24 from 55–64 cM, 3.41 from 68–88 cM, 3.18 from 95–116), chromosome 9 (3.49 from 154–167 cM), chromosome 17 (3.32 from 61–103 cM), and chromosome 22 (3.04 from 1–31 cM).

## Discussion

We have conducted the first extensive genetic study on pulse pressure in the Chinese population that covered both heritability estimation and gene mapping using a linkage approach. The significant heritability estimate confirms the genetic dissection on pulse pressure in the Chinese population, which is supported by our subsequent linkage analyses that identified multiple genomic regions harboring susceptibility loci for pulse pressure. Although each of the two approaches focused on different aspects of the genetic architecture of PP, overall, our significant results emphasize the genetic control over hemodynamic conditions.

By applying the GEE model to MZ and DZ twins separately, we were able to examine the effects of age, sex, and BMI on pulse pressure in Chinese twins. Highly significant effects of age, sex, and BMI were found in both MZ and DZ twins. Zhao et al. (2004) reported significant effects of dietary factors including salt intake on blood pressure differences between Chinese in the north (higher salt intake) and the south (lower salt intake). As a limitation of the study, salt intake was not measured and thus cannot be adjusted although individual adjustment on a population factor can only have limited effect within the same population. On the other hand, our models for gene mapping




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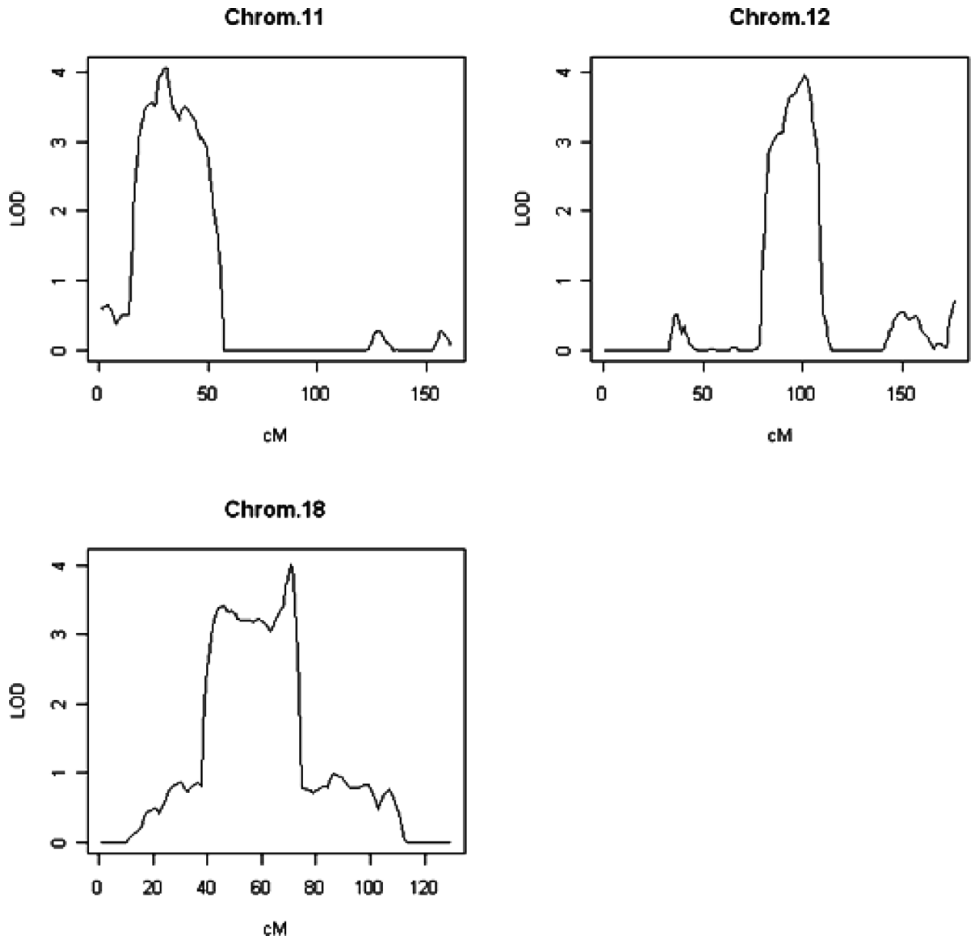


**FIGURE 3**

**The significant linkage peaks identified on chromosomes 11, 12, and 18 with lod score estimates of 4.06, 3.97, and 4.01.**

are based on intra-pair differences that match the effects of shared environmental factors (for example, family diet habits). It is encouraging to see that our results based on Chinese data are consistent with that from studies on other ethnic populations and give new support to the conclusion that the effects of these factors are cross-sectional (Skurnick et al., 2010).

Although low to modest heritabilities have been estimated in many different ethnic populations, to our knowledge, our twin-based study is the first to focus on PP in the Chinese population. Our heritability estimate of 0.45 is in agreement with recent studies reporting modest genetic effects in American Caucasians (DeStefano et al., 2004) and Africans (Bochud et al., 2005). Comparison of model performances suggested that the parsimonious AE model as the best model indicating the existence of additive genetic regulation on PP.

Three linkage peaks that reside separately on chromosomes 11, 12, and 18 have been found to show genome-wide significance (lod score $>$ 3.6) with their lod score estimates of around 4. It is surprising that among the three significant linkage peaks, two have been confirmed to show linkage in other populations. In a multipoint linkage scan performed on large and extended Utah pedigrees, Camp et al. (2003) reported linkage evidence on chromosome 12q at 109 cM which is covered by our linkage region on chromosome 12 in Figure 3. In another study based on American families, Bielinski et al. (2005) detected a significant linkage for PP on chromosome 18 at 71 cM, the most significant linkage in their study. It is interesting that this is exactly the location for our linkage peak on chromosome 18 (at 70.75 cM, Figure 3). Considering that there have been only a limited number of whole genome linkage scan on PP conducted in the literature, it is very unlikely that these results are coincidence.

Multiple suggestive linkages were detected by our linkage scan (Table 3). We assume that their trustworthiness increases with their lod score magnitudes. This seems to be reasonable considering the fact that four suggestive peaks with lod scores over 3 overlap with reported linkages in previous studies. The highest suggestive linkage peak on chromosome 1 lies in the same region where Sherva et al. (2007)




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reported a suggestive linkage at 215 cM. This region resides the G protein-coupled receptor gene, which is involved in the signaling cascades that regulate blood pressure during changes in cardiac output (Sherva et al., 2007). The highest suggestive linkage peak on chromosome 7 at 74.37 cM coincides with a linkage peak at 75cM reported by Bielinski et al. (2005), and is in the vicinity of a peak at 71 cM in the same region reported by DeStefano et al. (2004). The peak regions on chromosomes 17 and 22 cover linkage peaks for pulse pressure reported by Bielinski et al. (2005) (at 89 cM on chromosome 17 and 11 cM on chromosome 22). In addition, their study also detected linkages on chromosomes 1 (106 cM), 3 (172 cM), and 7 (75 cM) that are located in the vicinity of our suggestive regions. These results emphasize the importance of our suggestive linkages.

The performances of SNP and traditional microsatellite markers in linkage analysis have been compared by Schaid et al. (2004) and Evans and Cardon (2004) who consistently reported a higher information content from the dense map of SNP markers than the from the microsatellite markers spaced at 1 marker per ∼10 cM. Moreover, it was reported that linkage mapping using dense SNP markers identified more linkage peaks with more narrow widths than did traditional markers (Schaid et al., 2004). This conclusion coincides with our multiple suggestive scores with many of them overlapping with linkage peaks detected by published large studies.

Our identified significant and suggestive linkage peaks especially those overlapping closely with previous studies encourage further investigations with aim at identifying genetic variations that affect pulse pressure using more focused linkage and association approaches on large samples. Meanwhile, interpretation of the multiple suggestive linkages should be made with caution since the validity of these loci requires additional replication studies to be conducted particularly in the Chinese and Asian populations.

## List of Abbreviations

**AIC:** Akaike information criterion
**BMI:** body mass index
**DBP:** diastolic blood pressure
**DZ:** dizygotic
**GEE:** generalized estimating equation
**IBD:** identical-by-descent
**ICC:** intra-class correlation coefficient
**LD:** linkage disequilibrium
**MZ:** monozygotic
**PP:** pulse pressure
**SBP:** systolic blood pressure
**SNP:** single nucleotide polymorphism

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